SWAMI RAMANAND TEERTH MARATHWADA UNIVERSITY NANDED, MAHARASHTRA, INDIA

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"ISOLATION, BIOCHEMICAL AND BIOEVALUATION OF BIOFUNCTIONAL COLLAGEN PROPERTIES FROM FRESH WATER FISHES *LABEO ROHITA, CYPRINUS CARPIO AND PANGASIANODON HYPOPHTHALMUS*"

Research Proposal Submitted

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Format for submission of Ph.D. Synopsis / Work Plan for faculty of Science to be submitted In front of RRC

Abstract of proposed work plan/problem:

1. Introduction

2. Review of Literature

3. Objectives of Research / Proposed Hypothesis:

4. Methodology to be adopted:

5. Proposed work Plan

6. Year-wise Plan of work and targets to be achieved

7. References

Signature

Research Scholar

Signature

Research Supervisor

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Title of Ph.D. Work

ISOLATION, BIOCHEMICAL AND BIOEVALUATION OF BIOFUNCTIONAL COLLAGEN PROPERTIES FROM FRESH WATER FISHES *LABEO ROHITA*, *CYPRINUS CARPIO AND PANGASIANODON HYPOPHTHALMUS*

1. INTRODUCTION:

The word collagen has Greek origin, cola which means glue and genno means birth. Thus, collagen glues cells to form the basis for the body's tissues and organs. It comprises one-third of total protein in humans and accounts for three-quarters of the dry weight of skin. It is the most prevalent component of extracellular matrix (Brinckmann, 2005).

At present, there are at least 29 known types of collagen (Type-I-XXIX) (Veeruraj et al., 2015). It consists of three polypeptide chains, interwined to form collagen triple helix and it is mainly stabilized by intra- and inter-chain hydrogen bonding (Asghar and Henrickson, 1982). The triple helix, comprises a triplet sequence, Gly-X-Y, where Gly stands for amino acid glycine, X and Y are often amino acids proline and hydroxyproline (Shoulders and Raines, 2010). This structural organization of collagen can be lost by denaturation or melting. Different types of collagen are differently distributed in animal tissues.

Collagen is one of the most abundant biological macromolecules of extracellular matrix where it provides the major structural and mechanical support to tissues. Native collagens from different sources find applications in biomedical and pharmaceutical industries as well as cosmetics. Denatured collagen, known as gelatin, finds applications in the food and biomedical industries. Biomedical and pharmaceutical applications of collagen include the treatment of hypertension, urinary incontinence and pain associated with osteoarthritis, use in tissue engineering for implants in humans, inhibition of angiogenic diseases, such as diabetes complications, obesity, and arthritis (Hema G.S. 2015).

Commonly, the main sources for collagen production are pig skin, cattle skin and bone. The outbreak of BSE, TSE and foot and mouth diseases has resulted in justified anxiety amongst users of cattle collagen (Kittiphattanabawon et al., 2005). As a consequence, much attention has been paid to alternative sources of collagen, especially from fish skin and fish bone from the seafood processing industries. Gudmundsson and Hafsteinsson (1997); Choi and Regenstein (2000) have suggested that, the commercial use

of fish skin and bones, which are normally discarded, is a good waste management practice leading to additional economic benefit.

The objective of the study was to develop a method to isolate collagen from the raw material of **Water Fishes *Labeo rohita, Cyprinus carpio and Pangasianodon hypophthalmus*** using different acids (acetic, lactic and citric acid) and Biochemical properties were also evaluated.

2. REVIEW OF LITERATURE:

Collagen forms the major fraction of connective tissues such as skin, bone, tendon, the vascular system of animals and the connective tissue sheaths surrounding muscle (EA Foegeding et al., 1996).Type 1 collagen has been found as the major collagen in the skin, bone and fins of various fish species (S. Kimura et al., 1983). The physical and chemical properties of collagen differ depending on the tissues such as skin, swim bladder & the myocommata in muscle. Fish collagen is heat sensitive due to labile cross links as compared to mammals; the hydroxyproline content is lower, varying from 4-10% (K Sato et al., 1989). The amount of amino acids, especially hydroxyproline, depends on the environmental temperature in which the fish lives and it affects the thermal stability of the collagens and gelatins (G Balian et al. 1977) and (JK Jakhar *et al*. 2012). Collagens derived from fish species living in cold environments have lower contents of hydroxyproline and they exhibit lower thermal stability than those from fish living in warm environments. This is because of the involvement of hydroxyproline in inter-chain hydrogen bonding, which stabilizes the triple helical structure of collagen. Collagen film proved as a promising carrier for anticancer drug delivery system and ophthalmic drug delivery system because of its inertness, structural stability and good biocompatibility (E Mahdi *et al*, 2012) (HA Patel *et al* 2010) .

Fish skin, scales, and fins are composed of a highly ordered three-dimensional structure of the extracellular matrix, predominantly consisting of a type I collagen fibres and calcium-deficient hydroxyapatite (Zwarts et al., 2006).

Nutritionally, collagen and gelatine are poor sources of protein because they lack some of the essential amino acids in the proportions required by the human body. Collagen is used as a healing aid for burn patients, for bone reconstruction and for a wide variety of dental, orthopedic and cosmetic surgical purposes. The applicability of collagens from various sources depends on their thermal stability, which may directly correlate with the environment and body temperature of those species (Rigby, 1968).

Inherent chemical or physical collagen properties vary depending on the species from which collagen is isolated (Sadowska et al., 2003). Fish collagen can be produced from the discarded portion of the fish offal waste, such as the skin, scales, and fins, which are rich collagen sources (Dun et al., 2008).

3. OBJECTIVES OF THE STUDY:

Collagen is a fibrous protein that is primarily used in the pharmaceutical and biomedical industries. This study isolates and characterizes type-1 collagen from Indian major carps and Catfishes (scales, skin, and muscle).

1. To isolate and characterize collagen from three different species of fishes.
2. To develop fish collagen hydrolysate – optimization of the process parameters and characterization of collagen peptide.
3. To isolate acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC) from the bone, skin, fins and head of three different species of fishes.
4. Comparative study on composition of collagen from selected three different species of fishes.
5. To estimate the moisture, crude fat, crude protein and crude ash contents from selected freshwater fishes.
6. Explain the biochemical composition of collage from three different species of fishes.
7. Underline the meditional importance of fish collagen in human diet.
8. Justify that fish collagen is nutritionally important from the consumers' point of view.

4. METHODOLOGY TO BE ADOPTED

Sample collection:

Labeo rohita, Cyprinus crpio and Pangasianodon hypophthalmus scales, skin, muscles etc. raw material will collected of from a local fish market.

I. Collagen extraction:

Collagen will be extracted from selected three fish species i.e *Labeo rohita, Cyprinus crpio and Pangasianodon* using two methods: acid-solubilized collagen and pepsin-solubilized collagen.

- Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS– PAGE) will used to estimate the molecular weight of isolated collagen.
- Ultraviolet (UV)-visible spectrophotometry analysis will be used to confirm the collagen extracted (type-I collagen).
- The functional groups of isolated collagens will be identified using fourier transform infrared (FTIR analysis.
- The X-ray diffraction (XRD) technique will used to investigate the crystallinity of isolated collagen.
- The high-pressure liquid chromatography (HPLC) technique will used to study the amino acid composition.

II. Collagen characterization methods:

a) **Amino acid composition analysis by HPLC**: Amino acid composition analysis will carry out by using the method of Ishida et al. (1981).

b) **SDS-poly acryl amide gel electrophoresis:** Electrophoretic patterns of different species of collagens will analyse according to the method of Laemmli (1970).

c) **UV spectrophotometric analysis:** Collagen will dissolved in 0.5 M acetic acid to obtain a concentration of 1mg/ml. The solution after then subjected to UV–Vis measurement. Prior to measurement, the base line is set with 0.5 M acetic acid. The spectrum will obtained by scanning the wavelength in the range of 220–600 nm.

d) **FTIR analysis:** FTIR spectra of collagens were carried out as per the method described by Muyonga et al. (2004b)

III. **Proximate composition analysis:** The selected skin and the collagen extract will analyze for the proximate composition. The content of moisture, protein, fat and ash will analyze by the method of AOAC (2000).

a) **Determination of moisture:** The moisture content will determine according to the AOAC (2000)
b) **Determination of crude protein:** Micro- Kjeldahl distillation method will be used for crude protein determination.
c) **Determination of crude fat:** The ash content will measure by incineration of the sample according to AOAC, 2000).
d) Determination of ash: The ash content will measure by incineration of the sample according to AOAC, 2000).

IV. **Statistical analysis:** All experiments will done in triplicates. Mean values with standard deviations (SD) will report. Statistical calculations will performed in the SPSS 20 software package (IBM SPSS statistics, Version 20). Data analyses will performed using one way analysis of variance (ANOVA) with post-hoc multiple comparison analysis performed using Tukey's HSD test. P values ≤ 0.05 were considered statistically significant.

5. Significance of the Study

1. **Sustainable Resource Utilization**
   - Collagen isolation from freshwater fish utilizes underutilized resources, such as fishery by-products (skin, scales, and bones), which are often discarded as waste.
   - Promotes environmental sustainability by minimizing waste and reducing pollution.
2. **Economic Value Addition**
   - Adds value to the fish industry by converting by-products into high-value biofunctional materials.
   - Provides an economical alternative to traditional mammalian collagen sources.
3. **Unique Biochemical Properties**
   - Freshwater fish collagen exhibits unique biochemical characteristics, such as:
   - Distinct amino acid composition.
   - Lower denaturation temperatures, suitable for cold-temperature applications.
   - Serves as an excellent alternative for individuals allergic to bovine or porcine collagen.
4. **Biofunctional Benefits**
   - Demonstrates promising bioactivities, including:

- Antioxidant properties.
- Antimicrobial activity.
- These properties enhance its utility in medical, cosmetic, and pharmaceutical applications.

5. **Applications in Health and Industry**
   - Supports diverse applications, such as:
   - Wound healing and tissue engineering.
   - Drug delivery systems.
   - Development of cosmetic products.
6. **Alignment with Sustainability Goals**
   - Reduces dependency on overexploited marine resources.
   - Encourages a circular economy within the aquaculture and fish processing industries.
7. **Advancing Science and Innovation**
   - Paves the way for innovative bioengineering and healthcare applications.
   - Highlights the potential of untapped natural resources for a sustainable future

6. PROPOSED WORK PLAN:

First Year: Literature Review, Method Development, and Preliminary Studies

Literature Survey, Identification of selected fish species by Day Volume. Standardization of techniques and similarly pilot experiment show to be undertaken

Second Year:

- Experimental work on Collagen Extraction, Characteristics of fresh water three different fish species (Labeo rohita, Cyprinus carpio, and Pangasianodon hypophthalmus).
- Anyalyse the biofunctional properties of isolated collage.
- Compare collagen properties among the three fish species.
- Standardize protocols for proximate composition analysis (moisture, protein, fat, ash, etc.). and finalization of analyzed data etc.

Third Year:

Validation of Results

- Perform additional experiments or replicate studies to confirm findings.
- Address any gaps identified in earlier phases of the research.

Thesis Writing

- Compile all experimental data and analyses into a comprehensive thesis.
- Structure the thesis into chapters, including Introduction, Literature Review, Methodology, Results, Discussion, and Conclusion.
- All statistical calculation and graph making, Thesis writing and submission process

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